"METHOD FOR SELECTIVE INHIBITION OF HUMAN N-myc GENE
IN N-myc EXPRESSING TUMORS THROUGH ANTISENSE AND ANTI-
GENE PEPTIDE NUCLEIC ACIDS (PNA)"

*****

5 FIELD OF THE INVENTION

The present invention refers to sense and antisense
peptide nucleic acids (PNAs). The present invention
further refers to the use of said PNAs for preparing
drugs for treating genetic diseases.

10 PRIOR ART

It is known that antisense strategy can be validly
used to treat genetic or virus-related diseases.

According to antisense strategy, a RNA portion
complementary to a transcribed RNA region of a gene
15 can block the expression of transcribed RNA by
building a bond between complementary DNA and
transcribed RNA, so as to prevent the translation of
transcribed RNA.

In other words, short DNA sequences comprising 15-25
20 length bases are synthesized in complementary form and
are combined with portions of specific mRNAs of
viruses or of noxious origin that are present in tumor
cells.

The complementary portions thus built can block
25 translation directly.

Moreover, it is known about the use of antisense
strategy for preparing antisense drugs used in human
genetic therapy.

It is known about the use of antisense structures such
30 as for instance oligonucleotides.

However, in recent years the use of new antisense and
anti-gene structures has developed, such as peptide
nucleic acids (PNAs).

Peptide nucleic acids (PNAs) comprise analogs of

1

nucleic acids with neutral charge containing a pseudopeptide chain (backbone) instead of a common deoxyribose-phosphate structure.

Peptide nucleic acids (PNAs) are enzymatically more
5   stable if compared with oligonucleotide antisense structures.

Peptide nucleic acids can bind in a complementary way to DNA/RNA filaments, thus creating a hybrid PNA/DNA or PNA/RNA double helix structure, which are
10 thermodynamically more stable than homoduplexes.

Moreover, peptide nucleic acids can be synthesized through synthesis techniques commonly used for the synthesis of peptides.

In the light of the advantages disclosed above,
15 peptide nucleic acids (PNAs) represent an alternative approach for antisense gene therapy and are the most advantageous system for anti-gene strategy.

Furthermore, it has been shown that peptide nucleic acids are highly specific for target sequences and
20 enable to inhibit protein expression.

Therefore, peptide nucleic acids (PNAs) constitute a promising therapeutic approach for treating gene or virus-related diseases.

However, peptide nucleic acids (PNAs) have a drawback,
25 as for oligonucleotide antisense structures, i.e. they have a low capacity of getting through cell membrane.

In order to overcome such drawback, some researchers have tried to conjugate peptide nucleic acids with specific molecules so as to increase the effectiveness
30 of penetration of peptide nucleic acids through cell membrane.

Moreover, it is known that about 25-30% of untreated neuroblastomas show an amplification/overexpression of proto-oncogen N-*myc* associated with an advanced stage

2

of the disease, rapid progression and unfavorable
prognosis.

A neuroblastoma is a sarcoma originated by the
peripheral nervous system and consists of neuroblasts
5   (embryonic cells that will turn into nervous cells).
Neuroblastoma strikes children up to 10 years of age
and causes cranial and hepatic metastases.

N-myc expression in transgenic mice results in the
development of neuroblastomas.

10   In-vitro antisense inhibition of N-myc expression
reduces neuroblastoma proliferation and promotes the
differentiation of neuroblastoma tumor cells.

Inhibition has been accompanied until today both by
antisense oligonucleotide structures versus mRNA N-myc
15   and by the expression of carriers designed to generate
N-myc antisense RNA.

However, oligonucleotide antisenses have a drawback
consisting in their rapid degradation due to
nucleases.

20   Therefore, the identification of selective inhibitors
of N-MYC (protein) could have a high relevance for the
development of specific therapeutic agents with a
lower toxicity and a higher effectiveness for treating
N-myc expressing neuroblastomas.

25   As a consequence, there is the need for PNA sequences
that can inhibit or eliminate the synthesis of N-MYC
protein produced in tumors expressing said protein.

In particular, there is the need for PNA sequences,
conjugable if necessary, to be used in antisense and
30   anti-gene strategy so as to inhibit or eliminate the
synthesis of N-MYC protein.

In particular, there is the need for antisense PNA
sequences and anti-gene PNA sequences to be used for
preparing highly specific and effective drugs

(antisense and anti-gene drugs) for treating genetic
diseases or diseases caused by pathogenic viruses.
In particular, there is the need for selected peptide
nucleic acids that can bind messenger mRNA.

5   **AIMS OF THE INVENTION**

An aim of the present invention is to design and
select PNA sequences that can get through cell
membrane.

A further aim of the present invention is to design
10   and select PNA sequences to be used in antisense
strategy.

Another aim of the present invention is to design and
select PNA sequences to be used in anti-gene strategy.

Another aim of the present invention is to design and
15   select PNA sequences for selective inhibition of N-MYC
protein, for instance in human neuroblastoma cells.

Another aim of the present invention is to design and
select highly specific and effective PNA sequences for
preparing antisense and anti-gene drugs to be used for
20   treating genetic diseases.

These and other aims, as shall be evident from the
following detailed description, have been achieved by
the Applicant, who proposes an antisense strategy and
an anti-gene strategy based on the use of specific
25   peptide nucleic acids (PNAs) for inhibiting the
synthesis of N-MYC protein in tumors expressing said
protein, in particular in human neuroblastoma cells.

DESCRIPTION OF THE FIGURES

Figure 1 shows the result of an experiment in which
30   the inhibition effect of antisense PNA for 5'-UTR
sequence of N-myc transcript is measured on the growth
and proliferation of GI-LI-N and GI-CA-N cells. As
control, the inhibition effect is also measured with
an antisense PNA for 5'-UTR sequence of N-myc

4

transcript which has three mutation sites introduced
into it. The inhibition effects are measured by
collecting and counting the cells and measuring their
vitality by using colorimetric exclusion method
5   (tryphan blue dye) at 24, 48 and 72 hours.
Figure 2 shows the inhibition effect of treatment with
10μM sense anti-gene PNA in the proliferation of GI-
LI-N and IMR-32 cells with amplified N-myc gene
expression after 48 hours; while conversely 10μM
10 antisense anti-gene PNA do not show any inhibition
effect.
The inhibitory effect of sense anti-gene PNA is also
compared between IMR32 cells and GI-ME-N cells, which
shows how the amplification/overexpression of N-myc
15 gene is the factor that allows sense anti-gene PNA to
inhibit growth.
DESCRIPTION OF THE INVENTION
Therefore, a first object of the present invention
consists in PNA sequences having the characteristics
20 as in the appended independent claim.
Another object of the present invention consist in a
process to prepare the PNA sequences having the
characteristics as in the appended independent claims.
Another object of the present invention consists in
25 using said PNA sequences for treating genetic
diseases, whose characteristics are listed in the
appended independent claim.
Other preferred embodiments are listed in the appended
dependent claims, although without limiting the object
30 of the present invention.
In a preferred embodiment, the Applicant uses PNA
sequences for selective inhibition of N-MYC protein in
human neuroblastoma cells.
In order to show the effectiveness of the peptide

5

nucleic acids selected by the Applicant, the latter
has carried out experimental tests by selecting four
neuroblastoma cell lines: GI-LI-N, IMR-32 where N-*myc*
gene is amplified and overexpressed; and GI-CA-N, GI-
5   ME-N where N-myc gene is not amplified and is not
expressed.
Surprisingly, the Applicant has found out that the
antisense peptide nucleic acids selected by the
Applicant can get through cell membrane without using
10 a carrier.
Furthermore, the Applicant has surprisingly found out
that the inhibition effect, due to antisense and
anti-gene PNAs, on the synthesis of N-MYC protein is
highly selective and specific and has an anti-
15 proliferating effect.
Moreover, the stop of the growth of human
neuroblastoma GI-LI-N cells with amplified N-myc gene,
after the use of antisense PNAs, is directly followed
by cell differentiation or apoptosis (programmed cell
20 death).
Advantageously, peptide nucleic acid (PNA) comprises
12 to 24 nucleotide bases. Said peptide nucleic acid
is complementary to the sense or antisense filament of
human N-myc gene.
25 Preferred PNAs as described below and disclosed by way
of example below, are not however to be regarded as
limiting the present invention. As a matter of fact,
other types of PNA can also be carried out, by
suitably modifying their structure, so as to improve
30 their effectiveness and make them more specific and
suitable to various therapeutic needs. Also these
possible variants therefore fall within the framework
and aims of the present invention.
In a first embodiment, peptide nucleic acid is

complementary to the sense filament of human N-myc gene and is referred to as antisense PNA.

In a second embodiment, peptide nucleic acid is complementary to the antisense filament of human N-myc gene and is referred to as sense PNA.

The Applicant has designed an antisense peptide nucleic acid PNA (bp 135-150: 5'-TCCACCCAGCGCGTCC-3', genbank accession number M13241) that is complementary to only one sequence in 5'-UTR region of N-myc gene so as to inhibit an attack with the ribosome.

In order to assess how specific the activity of antisense PNA is, a mutated PNA containing the substitution of three bases has been designed (5'-CCCACTCAGCGCGCCC-3').

Antisense or sense PNA can be conjugated with a carrier that can get through the nuclear membrane of target cells, i.e. of tumor cells expressing N-myc gene.

Preferably, said carrier is conjugated in 3' position to PNA sequence.

In a preferred feature of the invention, said carrier consists of suitable peptide sequences deriving from appropriate proteins.

Said proteins are of various origin; for instance, they can derive from different types of viruses.

By way of absolutely non-limiting example, said proteins can be preferably selected among:

- Nuclear localization signal (NLS), from SV40 virus: the carrier consists of a peptide sequence PKKKRKV;
- Penetratin, from antennapedia; the carrier consists of a peptide sequence RQIKIWFQNRRMKWKK;
- Transportan: the carrier consists of a peptide sequence GWTLNSAGYLLGKINLAALAKKIL;
- Retro-inverso penetratin: the carrier consists of a

7

peptide sequence (D)-KKWKMRRNQFWVKVQR;
- TAT protein, from HIV virus: the carrier consists of a peptide sequence GRKKRRQRRRPPQ;
- TAT protein, from HIV virus: the carrier consists of a peptide sequence YGRKKRRQRRR.

5   Other peptide sequences to be used preferably as carriers can be selected for instance among the following ones:
- MSVLTPLLLRGLTGSARRLPVPRAKIHSL;
10 - KFFKFFKFFK;
- KKKK.

The amino acids constituting said peptide sequences can be both in L and in DL configuration.

In another preferred feature of the invention, PNA is
15 conjugated with carriers selected among peptides comprising amino acids with D or L configuration, whereby said peptides are bound directly to PNA through a stable covalent bond or through a disulfur labile bond, which can then be opened by reduction.
20 Peptides comprising D-arginine are particularly preferred.

In a third preferred feature of the invention, PNA is conjugated with carriers having various structures, whereby said carriers are bound directly to PNA
25 through a stable covalent bond or through a disulfur labile bond, which can then be opened by reduction. Among these carriers, retinoic acid is particularly preferred.

Antisense PNA conjugated with a carriers shows an
30 anti-gene PNA activity. Among anti-gene PNAs, those which bind to the antisense filament of N-myc gene are referred to as sense anti-gene PNAs, whereas those which bind to the sense filament of N-myc gene are referred to as antisense anti-gene PNAs.

Sense anti-gene PNAs have proved particularly effective towards target cells.

The Applicant has also designed sense anti-gene PNA and antisense anti-gene PNA sequences (sense anti-
5   gene: bp: 1650-1655 5'-ATGCCGGGCATGATCT-3'; antisense anti-gene 5'-AGATCATGCCCGGCAT-3' genbank accession number M13241), which are complementary to a sequence of exon 2 N-myc gene. Said sequences have been conjugated in 3' with a nuclear localization signal
10 (NLS) deriving from SV40 virus, so as to help it to get through nuclear membrane. The carrier consists of a peptide sequence PKKKRKV.

In a preferred embodiment, antisense PNAs and sense anti-gene or antisense anti-gene PNAs according to the
15 present invention are used for preparing pharmaceutical compositions.

In the following, by mere way of example, a method for the synthesis of peptide nucleic acids (PNAs) according to the present invention on micromolar scale
20 10, purification and characterization is described:

50 mg of polystyrene resin functionalized with methylbenzhydrylammino groups (MBHA-PS) are treated with dichloromethane (DCM) for 1 hour so as to make the resin swell. The resin is then washed with 5%
25 diisopropylethylamine (DIPEA) in dimethylformamide (DMF), DCM, further 5% DIPEA in DMF and N-methylpyrrolidone (NMP). A solution containing 0.01 millimoles of the first N-Boc protected C-terminal PNA monomer (available on the market) in 125 microliters
30 of NMP, 0.0095 millimoles of hexafluorophosphate benzotriazolyluronium (HBTU) in 125 microliters of NMP, is prepared separately, and the two solutions are mixed together. 0.02 millimoles of DIPEA are added and the whole is let activate for 2 minutes, then the

9

solution containing the activated monomer is put into contact with the resin. The reaction goes on for 1 hour, then the resin is washed repeatedly with NMP. Unreacted sites are blocked with a solution of acetic

5 anhydride/pyridine/DMF in a ratio of 1:2:2 put into contact with the resin for 1 hour. The absence of reactive sites is checked through a Kaiser test. In case of non-negative Kaiser test, blocking procedure is repeated. The resin is then washed repeatedly with

10 NMP, then with 5% DIPEA in DMF, then with DMC. The resin is now bound to the first C-terminal monomer in a ratio of 0.2 millimoles/gram.
The procedure of chain lengthening consists, for every monomer to be inserted, in a cycle including: Boc

15 group de-protection, pre-activation and coupling, block of unreacted sites if present (capping). Such cycles are usually carried out by means of an automatic synthesizer (Applied Biosystem ABI 433A). The solutions used for the various steps are listed

20 below. De-protection: trifluoroacetic acid (TFA) / m-cresol 95:5; pre-activation and coupling: 0.05 millimoles of protected N-Boc PNA monomer and 0.048 millimoles of HBTU dissolved in 500 microliters of NMP and added with 0.1 millimoles of DIPEA; capping:

25 acetic anhydride:pyridine:NMP 1:25:25. Rhodaminated PNAs have been synthesized using a spacing molecule (Boc-aminoethoxyethoxyacetic acid) in the last-but-one cycle instead of PNA monomer, and rhodamine in the last cycle instead of PNA monomer.

30 PNAs thus synthesized have been separated from the resin by means of solution of trifluoromethanesulfonic acid (TFMSA):TFA:m-cresol:thioanisol 2:6:1:1 and precipitated with the addition of ethyl ether to the separation solution.

10

Raw PNAs thus obtained have been analyzed through LC-
MS (analytical column C18 250x4.6 mm, gradient elution
between water added with 0.2% formic acid and a
solution of water:acetonitril 60:40 added with 0.2%
5 formic acid, flow rate 1 ml/min. UV detector at 260 nm
and mass detector in positive ionization mode, range
150-1500 m/z). Purification has been carried out using
a system resembling the analytical one, though using a
semi-preparative column (250x10 mm). The identity of
10 the pure compound has always been confirmed by mass
spectrometry. Typical yield after purification: 30%.
Typical purity after purification: 90-95%.
In order to assess the ability of antisense PNAs and
of anti-gene PNAs to get into human neuroblastoma
15 cells and to analyze the subsequent intracellular
localization, the Applicant has used four cell lines
GI-LI-N and IMR-32, GI-CA-N and GI-ME-N- and has
treated them for 30 minutes to 24 hours with 20 µM of
antisense or sense PNA conjugated with rhodamine in
20 5'. Anti-gene PNAs were further conjugated with NLS in
3'.
The picture on the fluorescent microscope shows that
intracytoplasmatic fluorescence for 5'-UTR antisense
PNA (in cell lines GI-LI-N and GI-CA-N) and
25 intranuclear fluorescence for anti-gene PNAs (in cell
lines GI-LI-N and GI-ME-N) can already be measured 30
minutes after cell treatment with PNA. Maximum
intensity is achieved in 6 hours, then the level is
constant for 24 hours.
30 High intracytoplasmatic values of antisense PNA were
observed, whereas for anti-gene PNAs high intranuclear
values were observed.
Untreated cells only show a background intracellular
fluorescence after six hours.

11

In order to assess the effectiveness and specificity of the peptide nucleic acids selected by the Applicant, the latter has used the four cell lines described above.

5   In threefold tests using plates with 24 wells, 1.0 x 10^5 cells have been introduced into the first wells with 0.5 ml of RPMI1640 containing 10% of FBS and 2 mM of L-butanine.

Cells have been incubated for 24 hours so as to let 10   them adhere to the base of the wells.

Then, in order to assess the optimal concentration for cell growth inhibition, peptide nucleic acid has been added to GI-LI-N cells in concentration of 10, 20, 40 and 60 μM for 5′-UTR antisense PNA, whereas for sense 15   and antisense anti-gene PNAs in concentrations of 1, 2, 5, 10 and 20 μM on GI-LI-N and IMR-32 cells-

In order to assess the specificity and selectivity of the effect of peptide nucleic acids onto N-MYC protein, GI-LI-N cells have been treated with a 20   variant of 5′-UTR antisense peptide nucleic acid with three mutation sites incorporated therein, in a concentration of 20 μM (optimal concentration selected for such PNA), and GI-CA-N cells (which do not have any amplification of N-myc gene) have been treated 25   with 5′-UTR antisense PNA in a concentration of 20 μM.

In order to assess the specificity of sense and antisense anti-gene PNAs, GI-ME-N and GI-CA-N (in which N-myc gene is not amplified and is not expressed) have been treated with sense and antisense 30   anti-gene PNA in a concentration of 10 −M (optimal concentration selected for sense anti-gene PNA).

Then, in order to assess the effects of the treatments, cells have been collected and counted 24, 48 and 72 hours after treatment.

Cells counting and vitality has been determined using colorimetric exclusion method (tryphan blue dye).

The treatment with 20 μM antisense PNA in GI-LI-N cells with amplified N-myc gene expression, shows a
5   high inhibition of cell growth. The maximum inhibition effect is of 70% and is achieved 48 hours after treatment (Fig. 1).

Conversely, GI-CA-N neuroblastoma cells with non-amplified N-myc gene expression and not expressing N-
10 myc, do not show any inhibition effect in the tests carried out under the same conditions (Fig. 1).

Proliferation tests on GI-LI-N cells using an antisense PNA containing a sequences altered by the introduction of three mutation sites, have not shown
15 any inhibition effect (Fig. 1). This proves the selective and specific action of antisense PNA for 5'-UTR sequence of N-myc transcript.

The production of N-MYC (protein) was assessed using Western Blotting in GI-LI-N cell line after treatment
20 with 20 μM antisense PNA in 24, 48 and 72 hours. An evident reduction of the protein level after 24 hours has been found. Said reduction decreases after 72 hours.

A flow cytometric analysis on GI-LI-N cells 36 hours
25 after treatment with 20 μM of antisense PNA, shows that said PNA induces a cell accumulation in G_0/G_1 from 34% to 57% and decreases in G_2 and in S phase from 13 to 6% and from 53% to 37%, respectively.

Moreover, the number of cells in sub-G_1 phase with a
30 hypodiploic DNA content (lower number of chromosomes than diploid DNA, i.e. 2n) increases from 3 to 22%.

In order to assess the differentiation of GI-LI-N cells towards neuronal cells, said cell line has been treated with 20 μM antisense PNA, whereby morphologic

13

changes have been detected by means of microscopic
analysis.

Microscope assessment has been carried 36 and 48 hours
after GI-LI-N cell growth in the presence or absence

5   of 20 µM antisense PNA.

After 36 hours treated cells have a less uniform
distribution than control cells, and after 48 hours
they tend to form small cell aggregates.

No effect of growth inhibition has been found for GI-

10 CA-N cells, but said effects have been found in tests
made on GI-LI-N cells.

Advantageously, the PNAs according to the present
invention show a high degree of selectivity for the
target designed on 5'-UTR N-myc sequence.

15 As further confirmation, no inhibition effect has been
observed in cell vitality, in cell cycle and in the
amount of N-MYC protein also after treatment with 10
µM mutated antisense PNA. This further shows the
specificity of the effect of antisense PNA.

20 Anti-gene PNA: the treatment with 10 µM sense anti-
gene PNA in GI-LI-N and IMR-32 cells with amplified N-
myc gene expression, causes a high inhibition of cell
growth.

As a matter of fact, the maximum inhibition effect is

25 of 90% in GI-LI-N cells and of 80% in IMR-32 cells and
is achieved 48 hours after treatment (Fig. 2; C(a) and
D(b)).

Conversely, neuroblastoma GI-ME-N and GI-CA-N cells
with non-amplified and non-expressed N-myc gene, do

30 not show any inhibition effect in the tests made under
the same conditions (Fig.2; E(c)).

Proliferation tests on GI-LI-N and IMR-32 cells using
10 µM antisense anti-gene PNA have not shown any
inhibition effect (Fig. 2; C(x) and D(y)). This proves

14

that the action of sense anti-gene PNA is selective and specific for the antisense filament of N-myc gene, and that the action of transcription inhibition is likely to unfold itself through the stop of RNA
5   polymerase, which uses as template its own antisense filament.

The production of N-myc transcript was assessed before and after 48 hours treatment with 10 µM sense anti-gene PNA by amplification in PCR of cDNA obtained from
10   250 ng of mRNA of GI-LI-N cells. The following primers have been used: sense CGACCACAAGGCCCTCAGT (ExonE 2, bp 2366); antisense TGACCACGTCGATTTCTTCCT (Exon 3, bp 5095) (Genbank M13241). PCR has been carried out with 30 reaction cycles. The results have shown that in GI-
15   LI-N cells treated with sense anti-gene PNA, the PCR product of N-myc transcript cannot be detected, whereas it can easily be detected in untreated cells.

Advantageously, anti-gene PNAs according to the present invention are highly specific for N-myc
20   amplification/overexpression.

The presence of an amplification/overexpression of N-myc gene is the main characteristic distinguishing GI-LI-N, IMR-32 cell lines from GI-ME-N and GI-CA-N cell lines.

25   No effect of growth inhibition has been found for GI-ME-N and GI-CA-N cells, but said effects have been found in tests made on IMR-32 cells.

Advantageously, the anti-gene PNAs according to the present invention show a high degree of selectivity
30   for the target designed on exon 2 sequence of N-myc.

As a matter of fact, anti-gene PNA has a high inhibitory effect, since it interferes directly with PNA polymerase during the transcription in the antisense filament, whereas the complementary

antisense anti-gene PNA has a much lower effect,
likely due only to the steric interference with the
transcription protein complex.

In further tests on sense anti-gene PNA, the
5   production of N-MYC protein was assessed by using
Western Blotting in IMR-32 cell line after 3 hours of
treatment with 10 μM sense anti-gene PNA. A reduction
of 50% of protein level has been detected after 3
hours of treatment with sense anti-gene PNA.

10 A cytofluorimetric analysis in IMR-32 cells 24 and 48
hours after the treatment with sense anti-gene PNA in
a concentration of 10 μM, induced a cell accumulation
in G_0/G_1 (from 39% to 53% after 24 hours; from 31% to
53% after 48 hours) and decrease in G_2/M (from 17% to
15 6% after 24 hours; from 25% to 9% after 48 hours) and
S phase (from 45 to 41% after 24 hours; from 44% to
39% after 48 hours).

In order to assess how specific the activity of sense
anti-gene PNA is, a mutated PNA containing the
20 substitution of three bases has been designed (5'-
GTGCCGAGCATGGTCT-3').

No inhibition effect has been observed in cell
vitality, in cell cycle and in the amount of N-MYC
protein also after treatment with a concentration of
25 10 μM of mutated anti-gene PNA and under the same test
conditions used for sense anti-gene PNA. This proves
the specificity of the effect of sense anti-gene PNA.

The treatment with 10 μM sense anti-gene PNA has also
been carried out in HT29 cells (deriving from colon
30 carcinoma) and in HeLa cells (deriving from cervical
carcinoma) expressing N-myc gene.

The treatment causes a high inhibition of cell growth.
As a matter of fact, the maximum inhibition effect is
of 70% in HT29 cells 48 hours after treatment, and of

70% in HeLa cells 24 hours after treatment.

Proliferation tests on HT29 and HeLa cells using 10 µM mutated sense anti-gene PNA have not shown any inhibition effect. This proves that also in colon and cervical carcinomas expressing N-myc, there is an inhibition effect using sense anti-gene PNA, and that such action is selective and specific for the antisense filament of N-myc gene.

The PNAs according to the present invention are interesting for the development of PNA-based drugs for specific treatments and neuroblastomas expressing N-MYC protein.

Such PNAs can also be used for other types of tumors expressing N-MYC protein such as for instance retinoblastoma, medulloblastoma, neuroblastoma, glioblastoma, astrocytoma or lung small cell tumor, rhabdomyosarcoma, B-type acute lymphoblastic leukemias.

17